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Ionol Derivatives from *Euphorbia tirucalli*

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Abstract: A new ionol derivative, (2*R*,6*S*,9*S*)-2-hydroxy-3-oxo-*α*-ionol (**1**), together with three known analogues, (6*S*,9*S*)-6-hydroxy-3-oxo-*α*-ionol (**2**), (6*R*,9*S*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**3**) and (6*R*,9*R*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**4**), were isolated from the ethyl acetate extract of the aerial parts of *Euphorbia tirucalli*. Their structures were elucidated by means of extensive spectroscopic methods and comparison with the data reported in the literature. The absolute configuration of **1** was deduced by comparing experimental and calculated ECD spectra and ^{13}C NMR data. The ionol derivatives have been obtained for the first time from the genus *Euphorbia*.

Keywords: *Euphorbia tirucalli*; ionol derivatives; structure elucidation.

1. Introduction

Euphorbia is the largest genus of the family Euphorbiaceae, comprising of more than 2000 species in the world and over 80 species in China [1], many of which have been used in folk as traditional Chinese medicine for the treatment of skin diseases, edemas, etc [2]. Previous chemical investigations revealed that diterpenoids, triterpenoids, flavonoids, phenolic acids, tannins and other constituents exist in this genus [3-8]. *Euphorbia tirucalli*, widely cultivated in tropical and subtropical regions, and the north and south areas in China [9], has been used as a traditional medicine in Africa and Asia for purgation and treatment of neuropathic pain, rheumatism, and toothache [10]. Previous researches have shown that the latex of *E. tirucalli* is toxic and strongly excitant on the skin and mucous membrane because of highly unsaturated diterpene ester [11-15].Our previous research reported the constituents of non-diterpenoids from *E. tirucalli* [16].

As an ongoing program to access chemical diversity of *Euphorbia* and their biological effects, we carried out an investigation on the air-dried of *E. tirucalli*, as a result, a new ionol derivative, (2*R*,6*S*,9*S*)-2-hydroxy-3-oxo-*α*-ionol (**1**), along with three known analogues, (6*S*,9*S*)-6-hydroxy-3-

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oxo-*α*-ionol (**2**) [17], (6*R*,9*S*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**3**) [18] and (6*R*,9*R*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**4**) [18], were isolated from the ethyl acetate extract of the aerial parts of this plant. To the best of our knowledge, the ionol derivatives were firstly obtained from the genus *Euphorbia*, which provided new evidences for the chemical diversity of *Euphorbia* plants. In this paper, the isolation and structural elucidation of the new compound are presented.

2. Materials and Methods

2.1. General procedures

1D and 2D NMR spectra were recorded on a Bruker Avance III-600 and a Bruker AM-400 instruments with TMS as internal standard. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer with KBr pellets. Optical rotations were recorded using a Jasco P-1020 Polarimeter. ESI-MS spectra were recorded on a Waters Xevo TQ-S UPLC Triple Quadrupole Mass Spectrometer. HR-ESI-MS data were obtained on an Agilent G6230 Q-TOF mass instrument. Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Factory, China, 200–300 mesh), Sephadex LH-20 (Pharmacia Biotech Ltd., Sweden) and MCI gel (CHP 20P, Mitsubishi Corporation, Japan). Thin-layer chromatography (TLC) and preparative TLC were performed using precoated silica gel GF_{254} plates (Qingdao Marine Chemical Factory). Semipreparative HPLC was performed on a Hitachi Chromaster system (Hitachi, Ltd., Japan) equipped with an YMC-Triart C_{18} column (250 mm × 10 mm i.d., 5 μm, YMC Corporation, Japan), using a flow rate of 3.0 mL/min at a column temperature of 25 °C, and detection was performed with a DAD detector.

2.2 Plant Material

The aerial parts of *E. tirucalli* were collected in August 2009 from Xishuang Banna prefecture, Yunnan Province, People's Republic of China, and were identified by Prof. Yao-Wen Yang, Yunnan University of Traditional Chinese Medicine, where a voucher specimen (YTCM 20090803) has been deposited.

2.3 Extraction and Isolation

The air-dried and powdered aerial parts of *E. tirucalli* (4.8 kg) were extracted with 70% aqueous acetone (8 L × 3) at room temperature. The extracts were concentrated by rotary evaporator under reduced pressure to remove organic solvent. The aqueous residue was then partitioned with petroleum ether (4 × 1 L), EtOAc (4 × 1 L), and n-BuOH (4 × 1 L), sequentially. The EtOAc extract (64.0 g) was subjected to MCI gel (CHP 20P) CC using a gradient system of CH_3OH–H_2O (30:70, 50:50, 70:70, 90:10) to afford four fractions (Fr A−D).

Fraction B (7.0 g) was chromatographed on Sephadex LH-20 column eluted by MeOH to give two fractions (Fr B-1−2) based on TLC analysis. Fr B-1 (1.2 g) was subjected to column chromatography (CC) on silica gel (200−300 mesh) eluting with $CHCl_3$–$(CH_3)_2CO$ (30:1−4:1) to afford two fractions (Fr B-1-1−2). Fr B-1-1 (298.7 mg) was purified by Sephadex LH-20 column (MeOH− $CHCl_3$, 1:1), followed by semipreparative HPLC (MeOH–H_2O 27:73) to yield compound **2** (6.5 mg, t_R = 23.0 min), and by semipreparative HPLC (MeOH–H_2O 35:65) to yield compound **1** (2.6 mg, t_R = 29.2 min). Fr B-1-2 (125.4 mg) was chromatographed on Sephadex LH-20 column (MeOH−$CHCl_3$, 1:1), followed by semipreparative HPLC (MeOH–H_2O 40:60) to afford compounds **3** (4.2 mg, t_R = 23.0 min), and **4** (1.0 mg, t_R = 29.0 min).

3. Results and Discussion

Compound **1**, $[\alpha]_D^{26.5}$ +136.2 (*c* 0.01, MeOH), UV (MeOH) λ_{max} (log ε): 235 (3.08) nm which revealed the presence of conjugated system, obtained as colorless powder in MeOH. Its molecular formula was determined to be $C_{13}H_{20}O_3$ based on the HR-ESI-MS data (*m/z* 223.1337 $[M-H]^-$, calcd. 223.1340), corresponding to 4 degrees of unsaturation. Its IR spectrum showed absorption bands for hydroxyl group at 3423 cm^{-1} and conjugated carbonyl group at 1672 cm^{-1}. The ^1H NMR spectrum (Table 1) showed three 3H-singlets at δ_H 1.85, 0.96 and 0.81, and one 3H-doublet at δ_H 1.11. An olefinic bond proton at δ_H 5.84 (1H, br s) and two olefinic protons [δ_H 5.59 (1H, dd, *J* = 15.2, 8.6 Hz); 5.63 (1H, dd, *J* = 15.2, 5.1 Hz)] were also evident in the ^1H NMR spectrum. Additionally, the signals of two oxygenated methines [δ_H 4.14 (1H, br t, *J* = 5.6 Hz), 3.95 (1H, s)] were also observed. ^{13}C NMR spectrum of **1** displayed most upfield resonance due to a conjugated carbonyl at δ_C198.7, along with four signals due to olefinic carbons (δ_C 162.0, s; 139.8, d; 124.3, d; 123.7, d), four methyl carbon resonances including two geminal methyls at δ_C 24.6 (s), 20.8 (s), one allelic methyl at δ_C 22.9 (s), and one primary methyl at δ_C 23.9. Accordingly, **1** was presumably an oxygenated ionol derivative substituted by a hydroxyl group and a carbonyl function.

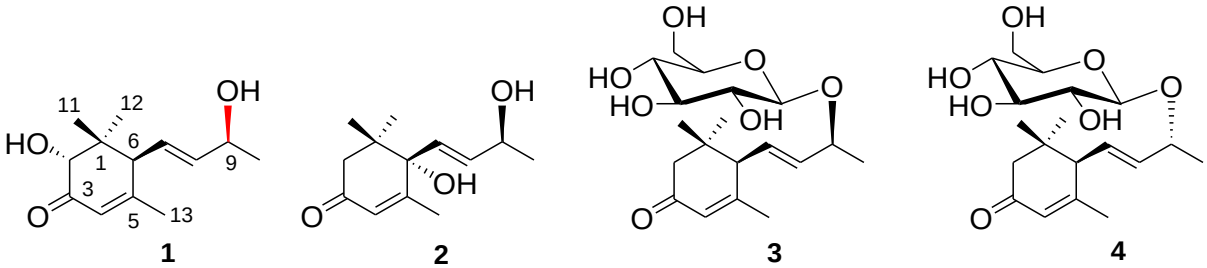

Figure 1. The chemical structures of compounds **1**-**4**.

The HMBC correlations (Fig. 2) from H-2 (δ_H 3.95) to C-1 (δ_C 41.1), C-3 (δ_C 198.7), and C-11 (δ_C 20.8), the COSY correlation of H_3-10 (δ_H 1.12) with H-9 (δ_H 4.14) and HMBC correlation of H-7 (δ_H 5.59) with C-9 (δ_C 66.2), respectively, allowed two hydroxyl groups to be located at C-2 and C-9, respectively and carbonyl function at C-3. The cross peaks between H_3-13 (δ_H 1.85) with C-4 (δ_C 123.7), C-5 (δ_C 162.0), and C-6 (δ_C 56.1) assigned the position of CH_3-13 and an enone system.

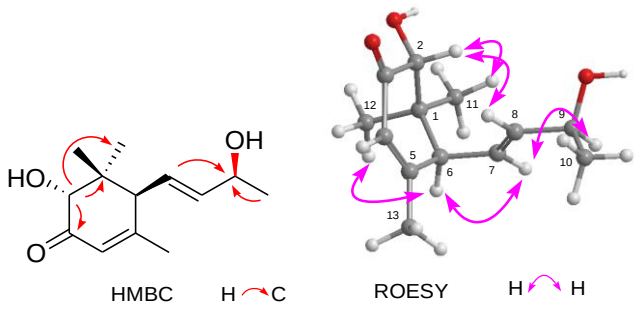

Figure 2. Key HMBC, and ROESY correlations of compound **1**.

The relative configuration in **1** was determined by the ROESY experiment (Fig. 2). The observed ROESY correlations: H-2 with H-8 and H-11, H-6 with H-7 and H-12, and H-7 with H-9 suggested that HO-2 and H-6 were of the same orientation on the α-face of the six-membered ring, HO-9 was the β-oriented, and the double bond was *E* configuration. Moreover, the amount of coupling constant (*J* = 15.2 Hz) between H-7 and H-8 substantiated *E* configuration of the double bond. In order to determine the absolute configuration of C-6 and C-9 the experimental and calculated ECD spectra predicted by TDDFT (Fig. 3) were carried out.

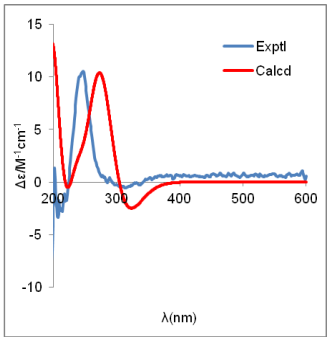

Figure 3. Calculated and experimental ECDs of **1** (red, calculated at the B3LYP-PCM/6-31G(d,p)//B3LYP/6-31G(d,p) level in CH_3OH; blue, experimental in CH_3OH).

The results (Fig. S2 and S6) revealed the structures of 1-A and 1-C were in accordance with the experimental spectra. Further comparing experimental and calculated ^{13}C NMR data (Fig. S22 and 23) gave the *S*-configuration of chiral center at C-9 ($\Delta\delta$ 3.3) rather than *R*-configuration ($\Delta\delta$ 4.6). Finally, the structure of compound **1** was assigned as (2*R*,6*S*,9*S*)-2-hydroxy-3-oxo-*α*-ionol.

The known compounds were identified to be (6*S*,9*S*)-6-hydroxy-3-oxo-*α*-ionol (**2**) [17], (6*R*,9*S*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**3**) [18] and (6*R*,9*R*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**4**) [18] by comparing their spectroscopic data with those in the literature.

Table 1. NMR data for compounds **1**–**4** (TMS as the internal standard, δ in ppm) [a]

No.	**1** [a, c]		**2** [b,c]	**3** [a,d]	**4** [a,d]
	δ_H *J* (Hz)	δ_C	δ_C	δ_C	δ_C
1		41.1 (s)	40.9 (s)	37.2 (s)	37.1 (s)
2	3.95 (1H, br s)	75.5 (d)	49.4 (t)	48.7 (t)	48.3 (t)
3		198.7 (s)	197.4 (s)	202.1 (s)	202.1 (s)
4	5.84 (1H, s)	123.7 (d)	125.5 (d)	126.4 (d)	126.1 (d)
5		162.0 (s)	164.4 (s)	165.7 (s)	165.9 (s)
6	2.67 (1H, br d, *J* = 8.6 Hz)	56.1 (d)	77.8 (s)	57.0 (d)	56.8 (d)
7	5.59 (1H, dd, *J* = 15.2, 8.6 Hz)	124.3 (d)	127.9 (d)	131.6 (d)	128.8 (d)
8	5.63 (1H, dd, *J* = 15.2, 5.1 Hz)	139.8 (d)	135.9 (d)	137.2 (d)	138.2 (d)
9	4.14 (1H, m)	66.2 (d)	66.1 (d)	74.9 (d)	77.0 (d)
10	1.12 (3H, d, *J* = 4.2 Hz)	23.9 (q)	24.1 (q)	22.3 (q)	21.0 (q)
11	0.81 (3H, s)	20.8 (q)	24.0 (q)	27.9 (q)	27.6 (q)
12	0.96 (3H, s)	24.6 (q)	23.1 (q)	28.2 (q)	28.0 (q)
13	1.85 (3H, s)	22.9 (q)	19.0 (q)	23.9 (q)	23.8 (q)
1'				101.1 (d)	102.4 (d)
2"				75.1 (d)	75.3 (d)
3"				78.4 (d)	78.1 (d)
4'				71.8 (d)	71.5 (d)
5'				78.4 (d)	78.0 (d)
6'				63.0 (t)	62.7 (t)

[a] NMR data were recorded at 600 MHz, [b] NMR data were recorded at 400 MHz,
[c] NMR data were recorded in DMSO-d_6 , [d] NMR data were recorded in CD_3OD.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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